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Epidermal Fin Tumors in the Gobiid Fish, *Bathygobius soporator.*

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(Plates I-V).

In their excellent review of the literature on neoplasias in cold-blooded vertebrates, Schlumberger & Lucké (1948) list thirteen reports of epitheliomas in fishes. Of these only one case involved the fins (Fiebiger, 1909), and the majority were epitheliomas of the lips or the oral mucosa.

In a collection of approximately 150 specimens of *Bathygobius soporator* (Cuvier & Valenciennes), three individuals were found to possess fin tumors of epidermal origin. The animals were collected in the vicinity of the Lerner Marine Laboratory, Bimini Island, B. W. I., during the months of July and August, 1949. A fourth tumorous specimen was sent to the writer by Dr. E. F. B. Fries, who collected it at the same locality in December, 1949.

Since the tumors in *Bathygobius* were found to be invasive and destructive of normal tissues, and since occurrence of epitheliomas in fishes has been infrequently described, the present report was undertaken.

The writer is indebted to the staff and facilities of the Lerner Marine Laboratory, Bimini Island, B. W. I., for the opportunity to collect and utilize this material. Dr. Ross F. Nigrelli, of the New York Aquarium, New York Zoological Society, kindly read and commented on the manuscript. The writer is also grateful to Dr. Eric F. B. Fries, of The City College, for one of the specimens used in this report.

GROSS MORPHOLOGY OF THE TUMORS.

For purposes of reference in this paper, the three tumorous individuals collected by the writer will be designated as "A," "B" and "C." The fourth specimen, collected by Dr. Fries, will be referred to as "D."

The sexes of the above animals were not determined, but, judging from their size, they may be presumed to have been sexually mature. They ranged from 6 to 7 cm. in standard length. During the time they were kept in the laboratory (about two weeks), they exhibited normal swimming and feeding behavior, and no function appeared impaired by the presence of the tumors on the fins.

The tumors all involve the fins and in each case more than one tumor is present. The growths are of different sizes and of varying degrees of invasiveness, so that they may be tentatively grouped in three classes on the basis of external, macroscopic appearance. A total of 12 tumors was examined.

1. The smallest tumors are visible as thickenings of the membranes at the distal ends of fin rays. The affected rays are slightly shortened and the membranes appear whitish and opaque. Five tumors of this type were found on three of the specimens examined.

2. The next stage of tumor formation appears as a distinct swelling of the epidermis into a rounded lump of tissue, colored light gray or white in life. Such overgrowths are small, covering an area of up to 4 sq. mm., and protruding about 0.3 to 0.5 mm. from the surface. The distal portions of several fin rays may be covered. Five tumors of this type were identified.

3. The largest tumors show considerable overgrowth, as well as complete destruction of the affected fin or portion of the fin. A compact, smooth-surfaced mass may be formed, or the surface may be rough and reddened. Two examples of these tumors were studied.

Following is a description of the tumors in the four specimens examined:

Specimen A.

Ventral Fins: These fins are normally fused into a single, median, sucker-like structure. In this specimen, the ventral fins are completely replaced by an irregular, lobed mass of tumor tissue (type 3, see above), measuring about 4 by 5 mm., and protruding about 2 mm. from the body. The surface of the tumor is rough and reddened.

Pectoral Fins: The ventral margins of both pectorals exhibit a visibly thickened epidermis covering the terminal portions of the four most ventral rays. An area of about 1 by 1.5 mm. is covered by these type 1 growths.

Caudal Fin: A tumor (type 2) covers the ends of the four most ventral rays. A slight overgrowth, 0.3 mm. in thickness, is present.

Specimen B.

Dorsal Fins: The anterior margin of each dorsal fin possesses a small, irregular, smooth-surfaced tumorous overgrowth, measuring approximately 1.5 mm. in diameter and 0.4 mm. in thickness (type 2 tumors). An epidermal thickening extends from each of these tumors distally and posteriorly over the first three spines of the anterior dorsal fin, and the first two rays of the posterior fin.

Left Pectoral Fin: The apex of the left pectoral fin shows a thickening over the distal 4 mm. of the five central, longest rays. An overgrowth (type 2), 2 mm. in diameter and 0.5 mm. in thickness, is present at the center of this area. Only the outer surface of the fin is affected.

Specimen C.

Pectoral Fins: Both pectorals exhibit tumors of type 1. The left pectoral possesses a thickened epidermis covering the five ventral rays. The right pectoral has a slight thickening at the apex, involving the distal 2 mm. of the two longest rays.

Specimen D.

Right Pectoral Fin: In the genus *Bathygobius*, the dorsal-most 5 to 8 fin rays are highly branched and lack a connecting membrane, forming a silk-like fringe of free rays. In this specimen, these free rays have been completely replaced on the right side by a compact, ovoid tumor, measuring 3 mm. in length, 0.8 mm. in width, and 1.7 mm. in depth (type 3 tumor). The tumor possesses a smooth surface and is attached at the dorsal margin of the base of the fin. An area of thickened epidermis extends ventrad over the bases of the two next fin rays.

Left Pectoral Fin: The ventral margin shows a slight epidermal thickening (type 1) covering an area of about 1 mm. by 1.5 mm.

Ventral Fins: The entire posterior margin of the fused ventral fins possesses a thickened epidermis. The fin rays appear slightly shortened. Three small overgrowths, each 0.7 mm. in diameter and 0.3 mm. in thickness, are present at the ends of three of the longest rays. These may be considered collectively as a type 2 tumor.

Histology of the Tumors.

For histological study, specimens A and B were fixed in Bouin's picro-formol, the others in 10% formalin. The fins were sectioned at 8 microns and stained with Harris' hematoxylin and eosin, or with a modified Masson trichrome technique. A red filter (Wratten A) was used for some of the photographs to bring out the blue-stained connective tissue and basement membranes produced by the latter technique. A green filter (Wratten B) was used for the rest of the photographs.

Normal Fin Epidermis (Figs. 1 & 2).

Over most of the surface of the fins, the epidermis varies from 40 to 70 microns in thickness, and consists of 10 to 20 layers of cells (Fig. 1). The outermost two or three layers are composed of squamous cells many of which are pycnotic (Fig. 2). Occasional gland cells are present here. Beneath the surface, the cells become less flattened, measuring 4 to 6 microns in thickness and 10 to 15 microns in width. The major thickness of the epidermis is composed of these cells. The basal portion of the epidermis consists of one or two layers of narrow columnar cells, ranging from 8 to 12 microns in height and 3 to 4 microns in width. A distinct basement membrane is present and a subjacent narrow, collagenous *stratum compactum* of the dermis. The latter is never more than 5 microns in thickness. The junction of the epidermis and the dermis forms a smooth or finely crenated margin.

The interior of the fin contains the fin rays, blood vessels, etc., within a loose connective tissue network. Muscle bundles are present beside each fin ray toward the base of the fin.

Type 1 *Tumors* (Figs. 3, 4, 8).

Macroscopically these growths appear as thickenings and opacities of the fin membrane, and histologically they are areas of epidermal hyperplasia with little or no invasion of subjacent tissues. Such a condition is found on the pectoral fins of specimens A and C and the left pectoral fin of Specimen D. The same type of hyperplasia is present around the margins of the type 2 and 3 tumors, where it gradates into the surrounding normal epidermal structure.

The hyperplastic epidermis varies from 70 up to 250 microns in thickness, and consists of 40 to more than 100 layers of cells (Fig. 3). The surface layers are similar to those of the normal epidermis, consisting of a few layers of squamous cells and occasional gland cells (Fig. 3). The surface, however, is irregular and wrinkled, with some of the cells cuboidal in shape. The basal layers are long columnar in the thinner regions and become cuboidal and more densely packed where the epidermis is thickest (Fig. 8). The major volume of the epidermis is composed of irregular polygonal cells 8 to 10 microns in size, with distinct cell membranes and clear cytoplasm (Fig. 4). The line of junction of the basal layers and the *stratum compactum* of the dermis is continuous but highly irregular, forming blunt indentations into the subjacent tissues (Fig. 3).

Type 2 *Tumors* (Figs. 7, 9, 10, 11).

These tumors represent the first stages of true invasion and overgrowth. They are present on the caudal fin of specimen A, the dorsals and left pectoral of specimen B (Fig. 7), and the ventral fin of specimen D.

A distinct surface coat is present, consisting of 3 to 5 layers of closely packed cuboidal cells (Fig. 10). The cells range from 4 to 8 microns in size. Few of these cells appear pycnotic or sloughing, and the entire

surface of the tumor is smoothly rounded. No gland cells were observed here.

A loose region is present beneath the surface coat, ranging from 20 to 90 microns in thickness (Figs. 9, 10). Most of the cells here are stellate in shape, and considerable intercellular space is visible. Some polygonal forms and larger vacuolated cells are present. These measure about 8 and 12 microns, respectively. The Masson trichrome stain showed no connective tissue fibers in this region.

The main mass of the tumor is derived from the basal layers. Epithelial pegs penetrate the *stratum compactum,* the subcutis and other internal fin structures (Fig. 9). At the points of penetration the pegs vary from 30 to 100 microns in thickness and are composed of densely packed cuboidal or polygonal cells averaging 5 microns in width (Fig. 11). The cores of the largest pegs possess small groups of 40 or 50 cells arranged in irregular circular masses. These groups may be classified as early stages in pearl formation.

Within the interior of the fin, the epithelial pegs ramify and send branches into muscle fascicles, around and between the lepidotrichia (Fig. 11). The center of the fin is swollen by the aggregation of the invading epithelial cords (Figs. 7, 9). Some of the pegs reach across to the opposite surface, the epidermis of which shows no signs of hyperplasia. In the center of the invaded region the muscle bundles are completely destroyed, but the fin rays are all intact (Fig. 11).

The invading cords possess a distinct basement membrane and a thin surrounding coat of collagenous fibers. Small blood vessels are present in the interstices between the network of cords (Figs. 9, 11).

Type 3 *Tumors.*

These tumors are characterized by extensive overgrowth and destruction of normal tissues. The two examples found differ somewhat from each other in structure, and therefore their histology will be described separately.

Right Pectoral Fin Tumor
of Specimen D (Fig. 6):

This growth forms a compact nodule attached to the dorsal, basal portion of the fin. The free pectoral rays are completely destroyed and the bases of the next two fin rays are invaded and their muscles destroyed. In cross-section, the tumor measures 0.8 mm. in width and 1.7 mm. in height, with the major portion consisting of an interwoven mass of epithelial pegs.

A surface coat of mixed cuboidal and squamous cells forms a smooth covering. One to five layers of these cells may be present. No gland cells are evident.

In the interstices of the complex meshwork of epithelial cords, numerous capillaries and small amounts of loose collagenous tissue are found. Capillaries are more numerous toward the surface of the tumor and are found within a tissue which appears similar to the sub-surface layer of polygonal, vacuolated or stellate cells described for the tumors of type 2.

Occasional incipient pearl formations are present within the centers of some of the epithelial cords. The pearls vary from 40 to 70 microns in diameter. Each pearl consists of a compact center of 10 to 40 polygonal cells. The cells average 3 to 5 microns in width, and the cores vary from 10 to 20 microns in diameter. Surrounding this core are 2 to 4 layers of tightly packed squamous cells and a loose area of irregularly concentric squamous and stellate cells.

Within the central mass of the tumor, the epithelial cords are so irregular and tightly packed that no average measurements can be given. They are similar in structure to those of the tumors of type 2, but basement membranes can be found only rarely. Toward the base, the tumor structure resembles that of type 2 more closely. A thickened epidermis and epithelial pegs invading the dermis and subcutis are present.

The fin rays below the base of the tumor are intact but the muscle associated with two of these rays is invaded or destroyed, in part, by invading tumor cords. Only the two rays just ventral to the tumor are so affected. Further ventrad, the epidermis over the next fin rays is thickened and possesses a structure like that of the tumors of type 1. No multi-nucleate, "giant" cells are found in this tumor.

Ventral Fin Tumor
of Specimen A (Figs. 5, 12-19):

This tumor is the largest and most invasive of all those studied. As described previously, the entire ventral fin complex is replaced by the growth.

In microscopic structure, this tumor is less compact and more vascularized than the one described from specimen D (Fig. 5). Numerous capillaries and sinusoids are present between the epithelial cords. Some of the sinusoids reach a diameter of 35 microns, while most of the smaller vessels are under 15 microns in width (Fig. 12).

An irregular surface coat of several layers of cuboidal cells covers the median ventral surface (Fig. 14). The surface coat is thinner, smoother, and composed of squamous cells toward the sides of the tumor. Some of the blood vessels are ruptured near the ventral median surface, and blood cells, along with surface epithelium, are found sloughing off in irregular masses of up to 60 to 100 cells. No gland cells are present.

Beneath the surface coat, there is a region of numerous small blood vessels, many of which are ruptured, and erythrocytes are visible within the loose meshwork of tumor tissue (Fig. 14). Although intact blood vessels exhibit some collagen at their surface, no collagenous fibers are found within this loose meshwork of cells. Some of the vessels contain numerous lymphocytes as well as

erythrocytes, but few lymphocytes or macrophages can be identified extravascularly.

Several large fluid-filled vesicles are present near the periphery of the tumor (Figs. 5, 13). These vary from 50 to 150 microns in diameter, and in each case a blood vessel is present in the center of these structures. The space around the blood vessel contains a loose granular network of precipitated material and a scattering of lymphocytes. Incipient vesicles may be found around many of the peripheral blood vessels and it is probable that they are edematous cysts formed by extravasation of blood fluids.

The major portion of the tumor consists of epithelial cords which form a less compactly interwoven mass than in the tumor of specimen D (Figs. 5, 12, 13). Many of the cords run parallel to each other, extending into the base of the tumor around the remnants of fin rays. The cords vary in size from narrow acuminate ones of 20 microns in width (Fig. 16), to broad, rounded shapes of up to 100 microns in width (Fig. 15). The centers of many of the cords contain incipient pearl formations (Figs. 18, 19) similar to those described for the tumor of specimen D above.

The surface of the cords possesses a 2- or 3-layered coat of cuboidal cells (Figs. 15, 16). These cells are about 3 to 5 microns in width, compactly arranged, and exhibit numerous mitotic figures. The cells in the centers of the cords are more loosely packed and frequently resemble mesenchymal cells in shape.

The basement membrane is distinct and continuous around most of the epithelial cords, but the ends of some show a breakdown of this membrane (Figs. 16, 17). This type of "flame" structure is present predominantly toward the base of the tumor and around the remnants of fin rays. The cells from these "flame" formations fan out and become stellate in shape (Fig. 17). How extensive a migration of these cells takes place is unknown. No evidence of metastasis was found on the fins in the vicinity of the tumors.

No multi-nucleate, "giant" cells can be identified in this or any of the previously described tumors.

Discussion.

Little is known of the actual genesis of the fin tumors of *Bathygobius*, but with the material thus far available, a sequence of the probable stages of growth of these neoplasms can be inferred. It is not known whether the tumors of type 1, which are simply epidermal hyperplasias, would, if given time, advance to type 2 or 3. Since the margins of the larger tumors are surrounded by such hyperplastic epidermis, however, it is probable that the growths arose from initial stages which were similar in structure to the tumors of type 1.

There are several characteristics of these tumors of *Bathygobius* which permit them to be allocated to the class of epitheliomas or epidermoid carcinomas. Chiefly, there is the formation of invading epithelial pegs and the destruction of fin rays and muscles. This was found in the tumors of type 2 and type 3. In the latter, there are areas where the basement membrane of the cords becomes broken down and flaring "flame" processes are formed. Such cords and flaring structures were considered as criteria of malignancy by Lucké & Schlumberger (1941) and Schlumberger & Lucké (1948), for lip epitheliomas in catfish (*Ameiurus nebulosus*).

Incipient pearl formation within the epithelial cords is present in both epitheliomas and papillomas of fishes. As pointed out by Schlumberger & Lucké (1948), true pearls cannot be formed in fish tumors since keratinization does not take place in the piscine integument. However, the presence of such incipient pearls is indicative of the resemblance of fish carcinomata to those of mammals.

Both pearl formations and epithelial cords have been reported as characteristics of epitheliomas in fishes, but with regard to other histological characteristics considerable variation exists from species to species.

In most cases the epithelial cords penetrate and destroy subjacent muscle and skeletal tissues. Johnstone (1923) described a tumor of the lower jaw of the whiting (*Merlangus merlangus*) which invaded a large portion of the mandible. Multiple tumors are also common, and as an extreme case, Christiansen & Jensen (1947) described multiple carcinomata covering almost the entire body surface in eels (*Anguilla anguilla*) from Danish waters.

The tumor mass is always highly vascularized, with numerous capillaries ramifying within the connective tissue interstices between the anastomosing tumor cords. The presence of larger and more numerous vessels toward the surface of the tumors was found in the present material and has been frequently reported. Lucké & Schlumberger (1941) observed that a localized, surface hyperemia precedes the formation of a tumor on the lips of catfish and that hyperemia persists throughout the development of the epithelioma. A similar hyperemic surface condition was reported by Fiebiger (1909) for a lip tumor in the tench (*Tinca tinca*), Johnstone (1923) for the jaw tumor of the whiting, and Christiansen & Jensen (1947) in an epithelioma of the eel. In this hyperemic region, sinusoid-like vessels were found in the type 3 tumors of *Bathygobius* similar to those described by Lucké & Schlumberger (1941) for *Ameiurus* tumors.

Other than this hyperemic condition, few signs of true inflammation have been reported. Lucké & Schlumberger (1941) found cells at the base of the catfish tumors which they tentatively identified as extravasated lymphocytes, and some leucocytes were found toward the surface of the ventral fin tumor in *Bathygobius*.

The epithelioma of the whiting (Johnstone, 1923) exhibited a condition in which the epithelial cords were short, instead of long and anastomosing. The main mass of the tumor possessed little continuity with the surface epidermis and consisted of numerous, isolated pearl formations, each surrounded by a capsule of connective tissue. Pearl formations, as found in *Bathygobius* tumors, and described by Fiebiger (1909), Lucké & Schlumberger (1941), and Schlumberger & Lucké (1948), were located within the epithelial cords and were surrounded by concentrically arranged, modified epithelial cells.

Johnstone (1923) also described a fusion of cells within the pearls into multi-nucleate structures, similar to those found by Fiebiger (1909) in fin tumors of the carp (*Cyprinus carpio*). Such multi-nucleate, "giant" cells were not observed in the *Bathygobius* tumors.

In the present material, the growth of the tumor was accompanied by the disappearance of epidermal gland cells. These cells are found in the normal epidermis and the type 1 tumors in *Bathygobius*, restricted to the surface layer. In species where mucoid and clavate gland cells are more numerous and more basally located, the epitheliomas exhibit these cells within the main tumor structure, in the centers of the epithelial cords. This has been reported by Fiebiger (1909) for *Tinca*, Lucké & Schlumberger (1941), and Christiansen & Jensen (1947). Williams (1928) described an epithelioma in the cod (*Pollachius virens*) in which gland cells were extremely numerous and actively secreting within the tumor mass, forming cyst-like cavities containing mucus. This latter tumor differed from the others in the possession of a heavy connective tissue stroma, as opposed to a thin, narrow network.

True metastases have never been reported in piscine epitheliomas, nor have any been identified in the present material. Multiple tumors evidently arise spontaneously. It is not known, however, how extensive a migration of cells takes place from the "flame" processes described here and by Lucké & Schlumberger (1941). Such structures as the tumor emboli found in blood vessels of catfish epitheliomas (Lucké & Schlumberger, 1941), and isolated pearl formations (Johnstone, 1923) may possibly be involved in the spread of the tumor.

Epitheliomas in fishes are frequently associated with some region where laceration or abrasion is likely to occur. Most of the reports listed by Schlumberger & Lucké (1948) give the lips, jaws and oral mucosa as the site of the tumor. Christiansen & Jensen (1947) state that the eel epithelioma usually begins its development around the mouth or on the head, which fact they correlate with the burrowing habits of the species. The most advanced growth described here in *Bathygobius* had replaced the sucker-like ventral fins which the goby uses in attaching itself to the substrate. No evidence of any infectious agent in the etiology of these tumors has been reported. Christiansen & Jensen (1947) attempted transmission of the disease with no success. They found some correlation between temperature changes and tumor incidence. Lucké & Schlumberger (1941), although successful in transplanting tumor tissue, were not able to correlate the incidence with any etiological agent.

Summary.

Four specimens of the gobiid fish, *Bathygobius soporator* (Cuvier & Valenciennes), collected at Bimini I., B. W. I., possess abnormal epidermal growths on the fins. The tumors vary from epidermal thickenings measuring 1 mm. by 1.5 mm. in area, to irregular, lobed overgrowths protruding from the body and completely replacing normal fin structure.

The smallest tumors consist of an epidermal hyperplasia. The second stage of tumor formation exhibits some overgrowth and a penetration of subjacent tissues by epithelial cords. The largest tumors are destructive of fin rays and muscles and exhibit characteristics of epitheliomas in the possession of invasive and flaring cords, numerous mitoses and incipient pearl formations. The tumors are highly vascularized and possess sinusoid-like vessels and fluid-filled vesicles toward the surface.

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EXPLANATION OF THE PLATES.

Plate I.

Fig. 1. Normal epidermis of pectoral fin. Masson stain. × 150. Green filter (Wratten B).

Fig. 2. Normal epidermis of pectoral fin. Masson stain. × 500. Green filter (Wratten B).

Fig. 3. Hyperplastic epidermis of left pectoral fin of Specimen C. Masson stain. × 150. Green filter (Wratten B).

Fig. 4. Surface of hyperplastic epidermis of left pectoral fin of specimen C. Masson stain. × 500. Green filter (Wratten B).

Plate II.

Fig. 5. Cross section of ventral fin tumor of specimen A. Masson stain. × 32. Green filter (Wratten B).

Fig. 6. Cross section of right pectoral fin tumor of specimen D. Masson stain. × 47. Green filter (Wratten B).

Fig. 7. Cross section of left pectoral fin tumor of specimen B. Masson stain. × 44. Green filter (Wratten B).

Plate III.

Fig. 8. Basal region of hyperplastic epidermis of left pectoral fin of specimen C. Masson stain. × 500. Green filter (Wratten B).

Fig. 9. Enlarged section of Fig. 7. Tumor of left pectoral fin of specimen B, showing epithelial pegs penetrating into subdermal region. Masson stain. × 120. Red filter (Wratten A). Red filter used to bring out dermal connective tissues.

Fig. 10. Enlarged section of Fig. 9. Surface of tumor of left pectoral fin of specimen B. Masson stain. × 500. Green filter (Wratten B).

Fig. 11. Enlarged section of Fig. 9. Interior of tumor of left pectoral fin of specimen B. Masson stain. × 500. Green filter (Wratten B).

Plate IV.

Fig. 12. Enlarged section of Fig. 5. Portion of ventral fin tumor of specimen A, showing surface vascularization and form of epithelial cords. Masson stain. × 100. Red filter (Wratten A). Red filter used to bring out connective tissues surrounding blood vessels and epithelial cords.

Fig. 13. Enlarged section of Fig. 5. Basal portion of ventral fin tumor of specimen A, showing epithelial cords and basal remnants of fin rays. Masson stain. × 120. Green filter (Wratten B).

Fig. 14. Enlarged section of Fig. 5. Surface of ventral fin tumor of specimen A, showing necrotic surface and loose, spongy subsurface tissue. Masson stain. × 500. Green filter (Wratten B).

Fig. 15. Enlarged section of Fig. 13. Terminus of an invading epithelial cord of ventral fin tumor of specimen A. Masson stain. × 500. Green filter (Wratten B).

Plate V.

Fig. 16. Enlarged section of Fig. 13. Epithelial cords of ventral fin tumor of specimen A, showing incipient breakdown of basement membrane. Masson stain. × 500. Red filter (Wratten A). Red filter used to bring out connective tissue and basement membranes.

Fig. 17. Enlarged section of Fig. 13. Terminus of epithelial cord of ventral fin tumor of specimen A, showing breakdown of basement membrane and formation of flaring "flame" process. Masson stain. × 500. Green filter (Wratten B).

Fig. 18. Incipient pearl formation in ventral fin tumor of specimen A. Masson stain. × 500. Green filter (Wratten B).

Fig. 19. Incipient pearl formation in ventral fin tumor of specimen A. Masson stain. × 500. Green filter (Wratten B).